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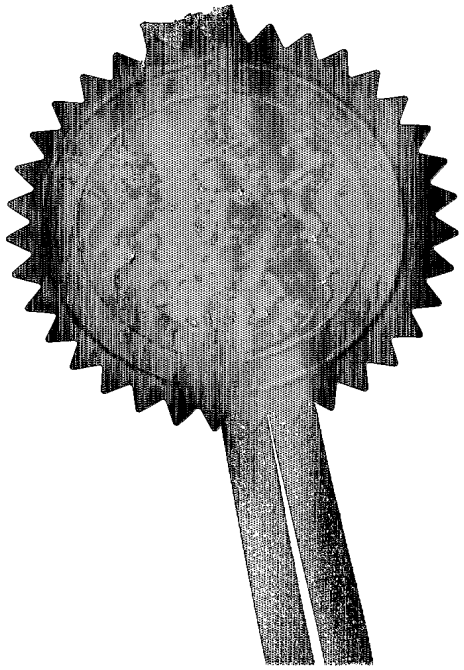

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GlaxoSmithKline Biologicals s.a.
Rue de l'Institut 89, B-1330 Rixensart, , Belgium
Ludwig Institute for Cancer Research, 605 3rd Avenue, New York, NY10158, USA
Belgian
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Vaccine

The present invention relates to improved vaccines and immunogenic compositions, and processes for the preparation of such vaccines and immunogenic compositions. In particular, the vaccines and immunogenic compositions of the present invention comprise, as an immunogen, IL-12 or IL-23 or subunits or components thereof, together with an adjuvant system comprising QS21 and 3 De-O-acylated monophosphoryl lipid A (3D-MPL).

IL-12 is a heterodimeric cytokine comprising the two subunits P40 and P35. IL-12 is produced mostly by phagocytic cells in response to bacteria, bacterial products, and intracellular parasites, and to some degree by B lymphocytes. In particular, IL-12 is produced by antigen presenting cells and instrumental in induction of TH-1 cell responses. IL-12 induces IFNγ from macrophages, natural killer (NK) cells and T lymphocytes, acts as a growth factor for activated NK cells and T lymphocytes, enhances the cytotoxic activity of NK cells, and induces cytotoxic T lymphocyte generation. IL-12 plays a central role in both the induction and magnitude of a primary Th1 response, and is essential to generate and sustain a sufficient number of memory/effector Th1 lymphocytes in vivo to mediate long-term protection against intracellular pathogens.

IFNγ is a cytokine produced by inflammatory T cells and NK cells.

IL-12 is thought to provide an important contribution to maintaining optimal resistance to intracellular pathogens such as Listeria, mycobacteria, Leishmania major or Toxoplasma. Additionally, individuals with IL-12-receptor deficiency have an increased risk of infection by such pathogens, although resistance to infection seems to increase with age.

IL-12 has been included in vaccine compositions, to assist in directing the immune response against antigens, particularly tumour associated antigens, contained in the vaccine compositions (WO98/57659).

IL-23 is a heterodimeric cytokine comprising the two subunits P40 (common to IL-12) and P19. Vaccine preparation is generally described in New Trends and Developments in Vaccines, edited by Voller et al., University Park Press, Baltimore, Maryland, U.S.A., 1978.

International patent application No. WO 95/17210 discloses an adjuvant emulsion system based on squalene, α-tocopherol, and polyoxyethylene sorbitan monooleate (TWEEN80), formulated with the immunostimulant QS21, optionally with 3D-MPL. This adjuvant formulation is a very potent inducer of a wide range of immune responses.

Saponins (reviewed in Lacaille-Dubois and Wagner 1996. Phytomedicine 2 363-386) are steroid or triterpene glycosides widely distributed in the plant and marine animal kingdoms. Saponins are known as adjuvants in vaccines for systemic administration. The adjuvant and haemolytic activity of individual saponins has been extensively studied in the art (Lacaille-Dubois and Wagner, supra). Immunologically active saponin fractions, *e.g.* Quil A, derived from the bark of the South American tree Quillaja Saponaria Molina are known in the art. Derivatives of Quil A, for example QS21 (an HPLC purified fraction derivative of Quil A), and the method of their production are disclosed in US Patent No.5,057,540. In addition to QS21 (also known as QA21) other fractions such as QA17 are also disclosed. The use of such saponins in isolation is disadvantageous because local necrosis, ie. localised tissue death, occurs at the injection site, thereby leading to pain and other health risks.

Other saponins which have been used in systemic vaccination studies include those derived from other plant species such as Gypsophila and Saponaria (Bomford et al., Vaccine, 10(9):572-577, 1992).

Oil-in-water emulsions per se are known in the art, and have been suggested to be useful as adjuvant compositions (EPO 399843).

Monophosphoryl lipid A (MPL) and derivatives thereof are known in the art. A preferred derivative is 3 De-O-acylated monophosphoryl lipid A (3D-MPL; UK Patent No. GB2220211), which can be prepared by methods taught in GB 2122204B. Chemically, 3-D MPL is a mixture of 3 De-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains and is manufactured by Ribi Immunochem Montana. A preferred form of 3 De-O-acylated monophosphoryl lipid A is disclosed in International Patent Application No. 92/116556.

In order for any oil-in-water composition to be suitable for human administration, the oil phase of the emulsion system has to comprise a metabolisable oil. The meaning of the term metabolisable oil is well known in the art. Metabolisable can be defined as "being capable of being transformed by metabolism" (Dorland's Illustrated Medical Dictionary, W.B. Sanders Company, 25th edition (1974)). The oil may be any vegetable oil, fish oil, animal oil or

synthetic oil, which is not toxic to the recipient and which is capable of being transformed by metabolism. Nuts, seeds, and grains are common sources of vegetable oils. Synthetic oils are also part of this invention and can include commercially available oils such as NEOBEE® and others. Squalene (2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-tetracosahexaene) is an unsaturated oil which is found in large quantities in shark-liver oil, and in lower quantities in olive oil, wheat germ oil, rice bran oil, and yeast. Squalene is a particularly preferred oil for use in this invention. Squalene is a metabolisable oil by virtue of the fact that it is an intermediate in the biosynthesis of cholesterol (Merck index, 10th Edition, entry no.8619).

Statement of Invention

The present invention provides an immunogenic composition comprising: (a) an immunogen comprising (a) an immunogenic component comprising (i) an immunogen comprising IL-12, IL-23, or a subunit or component thereof; and (ii) a carrier IL-12 or IL-23, or a subunit or component thereof and a carrier; and (b) an adjuvant.

The present invention is based on the surprising discovery that vaccination against an immunogen comprising IL-12 or IL-23 or a subunit or component thereof and a carrier, in combination with an adjuvant comprising one or more of cholesterol, oil-in-water emulsion, tocopherol, QS21 and 3 D-MPL, breaks tolerance to raise an immune response against IL-12 or IL-23 or subunit or component thereof. Further, the inventors have made the surprising discovery that such vaccination is extremely effective in the amelioration, treatment or prevention of several diseases.

The invention further relates to a vaccine composition comprising the immunogenic composition as described above in combination with a pharmaceutically acceptable excipient, adjuvant or carrier.

The present invention further provides a process for the manufacture of a vaccine composition comprising mixing the immunogenic composition as described herein with a pharmaceutically acceptable excipient, adjuvant or carrier.

The invention further relates to a method of preventing or treating a disease, in particular an autoimmune-implicated disease by administering to an individual at risk of these diseases an immunogenic composition or vaccine composition as listed above.

The invention further provides the use of an immunogen composition or vaccine composition according to the present invention which is capable of generating an immune response against IL-12 or IL-23, or a subunit or component thereof, in the manufacture of a vaccine for the treatment of a disease, in particular an autoimmune-implicated disease.

Detailed Description

The immunogenic composition of the present invention is suitably capable of stimulating an immune response to prevent or treat disorders including autoimmune-implicated diseases. The present invention may be used to treat disorders of mammals; preferably the mammal to be treated is human.

Immunogenic component

An immunogen according to the present invention is a substance suitably capable of stimulating an immune response to an immunogen which forms part of the immunogenic component, in vitro, ex vivo or in vivo. Preferably, the immune response is capable of being stimulated in vivo.

IL-12

The term "IL-12" is used herein to mean isolated naturally occurring human or other mammalian interleukin-12, or recombinant human or other mammalian IL-12. By isolated IL-12 is meant IL-12 substantially free of contaminants which may have been present at the beginning of an isolation process. By subunit of IL-12 is meant either of the two peptide subunits, P40 or P35 which comprise IL-12. By component of IL-12 is meant any fragment or epitope of IL-12 or subunit thereof capable of stimulating an immune response against IL-12, fragment or epitope of IL-12 or subunit thereof.

IL-23

The term "IL-23" is used herein to mean isolated naturally occurring human or other mammalian interleukin-23, or recombinant human or other mammalian IL-23. By isolated IL-23 is meant IL-23 substantially free of contaminants which may have been present at the beginning of an isolation process. By subunit of IL-23 is meant either of the two peptide subunits, P40 or P35 which comprise IL-23. By component of IL-23 is meant any fragment or epitope of IL-23 or subunit thereof capable of stimulating an immune response against IL-23, fragment or epitope of IL-23 or subunit thereof.

In one embodiment of the invention the subunit is P35 of IL-12 or P19 of IL-23. Most preferably, the subunit is P40 of IL-12 or IL-23. In a further embodiment, the immunogen comprises at least one surface or discontinuous epitope of one of the subunits of the present invention. Preferably, the immunogen comprises at least one surface epitope of P40. Preferably, the vaccine of the present invention comprising the subunit P40 is capable of stimulating an immune response against IL-12 or the subunit thereof and or IL-23 or the subunit thereof.

Carrier

A non-exhaustive list of carriers which may be used in the present invention includes: Keyhole Limpet Haemocyanin (KLH), serum albumins such as bovine serum albumin (BSA), ovalbumin (OVA), inactivated bacterial toxins such as tetanus or diphtheria toxins (TT and DT), or recombinant fragments thereof (for example, Domain 1 of Fragment C of TT, or the translocation domain of DT), the purified protein derivative of tuberculin (PPD).

In an embodiment of the invention a preferred carrier is Protein D from *Haemophilus influenzae* (EP 0 594 610 B1). Protein D is an IgD–binding protein from *Haemophilus influenzae* and has been patented by Forsgren (WO 91/18926, granted EP 0 594 610 B1). In some circumstances, for example in recombinant immunogen expression systems it may be desirable to use fragments of protein D, for example Protein D 1/3rd (comprising the N-terminal 100-110 amino acids of protein D (GB 9717953.5)).

Accordingly, preferred immunogens of the present invention comprise IL-12 or IL-23 a subunit or component thereof, presented in a recombinant expression system or conjugated to a carrier molecule, preferably a carrier protein, such that the recombinant expression system or the carrier protein provide T-cell help for generation of an immune response to the IL-12 or IL-23 subunit or component thereof. Particularly preferred immunogens comprise the P40 subunit conjugated or fused to a carrier protein to provide T-cell help for generation of an immune response to P40.

A preferred recombinant expression system for use in the present invention is HepB core. Preferred carrier molecules include TT, BSA, KLH, OVA and PADRE. TT and PADRE being particularly preferred.

In one embodiment of the present invention the immunogenicity of the peptides is enhanced by the addition of a "T-cell helper (Th) epitope" or T-helper epitope", which is a peptide able to bind to an MHC molecule and stimulate T-cells in an animal species. Preferably, the T-

helper epitope is a foreign or non-self epitope. Preferred T-cell epitopes are promiscuous epitopes, ie. epitopes that bind to a substantial fraction of MHC class II molecules in an animal species or population (Panina-Bordignon et al, *EJI.* 1989, 19:2237-2242; Reece et al, *JI* 1993, 151:6175-6184). The immunogenic components of the present invention may, therefore, comprise an immunogen comprising IL-12 or IL-23 or a subunit or component thereof and promiscuous Th epitopes either as chemical conjugates or as purely synthetic peptide constructs. The immunogen is preferably joined to the Th epitopes via a spacer (e.g., Gly-Gly) at either the N- or C-terminus of the immunogen. In order for the immunogenic components of the present invention to be sufficiently clinically effective, it may be necessary to include several foreign T-cell epitopes. The immunogenic components may comprise 1 or more promiscuous Th epitopes, and more preferably between 2 to 5 Th epitopes.

A Th epitope is a sequence of amino acids that comprise a Th epitope. A Th epitope can consist of a continuous or discontinuous epitope. Hence not every amino acid of Th is necessarily part of the epitope. Th-epitopes that are promiscuous are highly and broadly reactive in animal and human populations with widely divergent MHC types (Partidos et al. (1991) "Immune Responses in Mice Following Immunisation with chimaeric Synthetic Peptides Representing B and T Cell Epitopes of Measles Virus Proteins" J. of Gen. Virol. 72:1293-1299; US 5,759,551). The Th domains that may be used in accordance with the present invention have from about 10 to about 50 amino acids, and preferably from about 10 to about 30 amino acids. When multiple Th epitopes are present, these may all be the same (ie the epitopes are homologous) or a combination of more than one type of epitope may be used (ie the epitopes are heterogeneous).

Th epitopes include as examples, pathogen derived epitopes such as Hepatitis surface or core (peptide 50-69, Ferrari et al., J.Clin.Invest, 1991, 88, 214-222) antigen Th epitopes, Pertussis toxin Th epitopes, tetanus toxin Th epitopes (such as P2 (EP 0 378 881 B1) and P30 (WO 96/34888, WO 95/31480, WO 95/26365), measles virus F protein Th epitopes, Chlamydia trachomatis major outer membrane protein Th epitopes (such as P11, Stagg et al., Immunology, 1993, 79, 1-9), Yersinia invasin, diphtheria toxoid, influenza virus haemagluttinin (HA), and P.falciparum CS antigen.

Other Th epitopes are described in the literature, including: WO 98/23635; Southwood et al., 1998, J. Immunol., 160: 3363-3373; Sinigaglia et al., 1988, Nature, 336: 778-780; Rammensee et al., 1995, Immunogenetics, 41: 4, 178-228; Chicz et al., 1993, J. Exp. Med.,

178:27-47; Hammer et al., 1993, Cell 74:197-203; and Falk et al., 1994, Immunogenetics, 39: 230-242, US 5,759,551; Cease et al., 1987, PNAS 84, 4249-4253; Partidos et al., J.Gen.Virol, 1991, 72, 1293-1299; WO 95/26365 and EP 0 752 886 B. The T-cell epitope can also be an artificial sequence such as a Pan D-R peptide "PADRE" (WO 95/07707).

The foreign T-cell epitope is preferably selected from the group of epitopes that will bind to a number of individuals expressing more than one MHC II molecules in humans. For example, epitopes that are specifically contemplated are P2 and P30 epitopes from TT (Panina–Bordignon Eur. J. Immunol 1989 19 (12) 2237). In a preferred embodiment the heterologous T-cell epitope is P2 or P30 from TT.

The P2 epitope has the sequence QYIKANSKFIGITE and corresponds to amino acids 830-843 of the Tetanus toxin.

The P30 epitope (residues 947-967 of Tetanus Toxin) has the sequence FNNFTVSFWLRVPKVSASHLE; the FNNFTV sequence may optionally be deleted.

Other universal T epitopes are derivable from the circumsporozoite protein from Plasmodium falciparum – in particular the region 378-398 having the sequence DIEKKIAKMEKASSVFNVVNS (Alexander J, (1994) Immunity 1 (9), p 751-761).

Another epitope which may be used is derived from Measles virus fusion protein at residue 288-302 having the sequence LSEIKGVIVHRLEGV (Partidos CD, 1990, J. Gen. Virol 71(9) 2099-2105).

Yet another epitope which may be used is derived from hepatitis B virus surface antigen, in particular amino acids, having the sequence FFLLTRILTIPQSLD.

Another set of epitopes which may be used is derived from diphtheria toxin. Four of these peptides (amino acids 271-290, 321-340, 331-350, 351-370) map within the T domain of fragment B of the toxin, and the remaining 2 map in the R domain (411-430, 431-450):

PVFAGANYAAWAVNVAQVI
VHHNTEEIVAQSIALSSLMV
QSIALSSLMVAQAIPLVGEL
VDIGFAAYNFVESII NLFQV

QGESGHDIKITAENTPLPIA
GVLLPTIPGKLDVNKSKTHI

(Raju R., Navaneetham D., Okita D., Diethelm-Okita B., McCormick D., Conti-Fine B. M. (1995) Eur. J. Immunol. 25: 3207-14.)

Alternatively the immunogen may be directly conjugated to liposome carriers, which may additionally comprise immunogens capable of providing T-cell help.

Preferably the ratio of immunogen to carrier molecules is in the order of between about 1:10 to about 20:1. Preferably each carrier should carry between about 3 to about 15 molecules of immunogen. In an alternative preferred embodiment, each immunogen should carry between about 3 to about 15 carrier molecules. In an embodiment of the invention in which the carrier is PADRE or a Tetanus peptide, the ratio of immunogen to carrier peptides is between about 1:5 to about 1:10.

Conjugation or fusion protein

The immunogen of the present invention may be coupled to the carrier by a method of conjugation well known in the art. Thus, for example, for direct covalent coupling it is possible to utilise a carbodiimide, glutaraldehyde or (N-[γ-maleimidobutyryloxy] succinimide ester, utilising common commercially available heterobifunctional linkers such as CDAP and SPDP (using manufacturers instructions). After the coupling reaction, the conjugate immunogen can easily be isolated and purified by means of a dialysis method, a gel filtration method, a fractionation method etc. Conjugates formed by use of gluteraldehyde or maleimide chemistry are preferred for use in the present invention. Maleimide chemistry being particularly preferred.

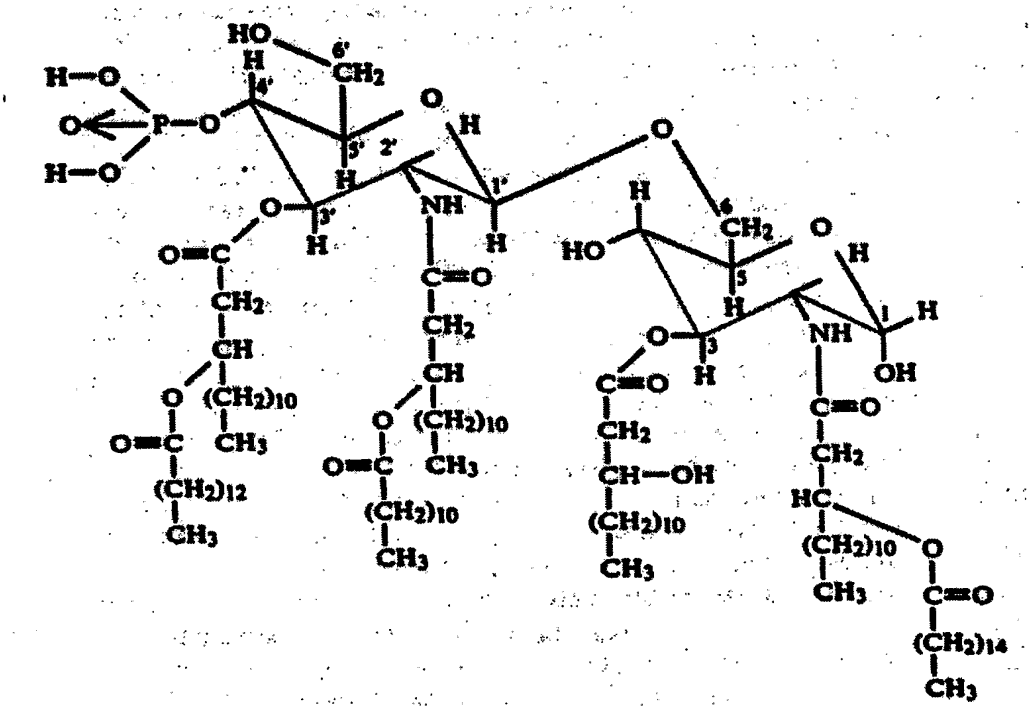
Alternatively, the immunogen is coupled to the carrier in the context of a recombinant fusion molecule. For example, EP 0 421 635 B describes the use of chimaeric hepadnavirus core antigen particles to present foreign peptide sequences in a virus-like particle. As such, fusion molecules may comprise immunogen of the present invention presented in chimaeric particles consisting of e.g. hepatitis B core antigen. Alternatively, the recombinant fusion proteins may comprise immunogen and NS1 of the influenza virus. For any recombinantly expressed protein which forms part of the present invention, the nucleic acid which encodes said protein also forms an aspect of the present invention.

Preferably, the conjugate or fusion protein is substantially biologically inactive, such that it is substantially unable to signal through IL-12 or IL-23 receptors.

Adjuvant

The vaccine or composition according to the invention comprises an adjuvant or immunostimulant, with preferred adjuvants including (but not limited to) detoxified lipid A from any source and non-toxic derivatives of lipid A, saponins and other reagents capable of stimulating a TH1 type response.

It has long been known that enterobacterial lipopolysaccharide (LPS) is a potent stimulator of the immune system, although its use in adjuvants has been curtailed by its toxic effects. A non-toxic derivative of LPS, monophosphoryl lipid A (MPL), produced by removal of the core carbohydrate group and the phosphate from the reducing-end glucosamine, has been described by Ribi et al (1986, Immunology and Immunopharmacology of bacterial endotoxins, Plenum Publ. Corp., NY, p407-419) and has the following structure:



A further detoxified version of MPL results from the removal of the acyl chain from the 3-position of the disaccharide backbone, and is called 3-O-Deacylated monophosphoryl lipid A (3D-MPL). It can be purified and prepared by the methods taught in GB 2122204B, which reference also discloses the preparation of diphosphoryl lipid A, and 3-O-deacylated variants thereof.

A preferred form of 3D-MPL is in the form of an emulsion having a small particle size less than 0.2μm in diameter, and its method of manufacture is disclosed in WO 94/21292. Aqueous formulations comprising monophosphoryl lipid A and a surfactant have been described in WO9843670A2.

The bacterial lipopolysaccharide derived adjuvants to be formulated in the compositions of the present invention may be purified and processed from bacterial sources, or alternatively they may be synthetic. For example, purified monophosphoryl lipid A is described in Ribi et al 1986 (supra), and 3-O-Deacylated monophosphoryl or diphosphoryl lipid A derived from *Salmonella sp.* is described in GB 2220211 and US 4912094. Other purified and synthetic lipopolysaccharides have been described (Hilgers *et al.*, 1986, *Int.Arch.Allergy.Immunol.*, 79(4):392-6; Hilgers *et al.*, 1987, Immunology, 60(1):141-6; and EP 0 549 074 B1). A particularly preferred bacterial lipopolysaccharide adjuvant is 3D-MPL.

Accordingly, the LPS derivatives that may be used in the present invention are those immunostimulants that are similar in structure to that of LPS or MPL or 3D-MPL. In another aspect of the present invention the LPS derivatives may be an acylated monosaccharide, which is a sub-portion to the above structure of MPL.

Saponins are taught in: Lacaille-Dubois, M and Wagner H. (1996. A review of the biological and pharmacological activities of saponins. Phytomedicine vol 2 pp 363-386). Saponins are steroid or triterpene glycosides widely distributed in the plant and marine animal kingdoms. Saponins are noted for forming colloidal solutions in water which foam on shaking, and for precipitating cholesterol. When saponins are near cell membranes they create pore-like structures in the membrane which cause the membrane to burst. Haemolysis of erythrocytes is an example of this phenomenon, which is a property of certain, but not all, saponins.

Saponins are known as adjuvants in vaccines for systemic administration. The adjuvant and haemolytic activity of individual saponins has been extensively studied in the art (Lacaille-Dubois and Wagner, *supra*). For example, Quil A (derived from the bark of the South American tree Quillaja Saponaria Molina), and fractions thereof, are described in US 5,057,540 and "Saponins as vaccine adjuvants", Kensil, C. R., *Crit Rev Ther Drug Carrier Syst*, 1996, 12 (1-2):1-55; and EP 0 362 279 B1. Particulate structures, termed Immune Stimulating Complexes (ISCOMS), comprising fractions of Quil A are haemolytic and have been used in the manufacture of vaccines (Morein, B., EP 0 109 942 B1; WO 96/11711; WO

96/33739). The haemolytic saponins QS21 and QS17 (HPLC purified fractions of Quil A) have been described as potent systemic adjuvants, and the method of their production is disclosed in US Patent No.5,057,540 and EP 0 362 279 B1. Other saponins which have been used in systemic vaccination studies include those derived from other plant species such as Gypsophila and Saponaria (Bomford *et al.*, Vaccine, 10(9):572-577, 1992).

An enhanced system involves the combination of a non-toxic lipid A derivative and a saponin derivative particularly the combination of QS21 and 3D-MPL as disclosed in WO 94/00153, or a less reactogenic composition where the QS21 is quenched with cholesterol as disclosed in WO 96/33739.

A particularly potent adjuvant formulation involving QS21 and 3D-MPL in an oil-in-water emulsion is described in WO 95/17210 and is a preferred formulation.

Preferably the vaccine additionally comprises a saponin, more preferably QS21.

Preferably the formulation additionally comprises an oil-in-water emulsion. In one embodiment of the present invention, the adjuvant consists of an oil-in-water emulsion.

Preferred oil-in-water adjuvant emulsions are described in PCT application no. WO 95/17210. These have a high ratio of squalene : saponin (w/w) of 240:1. Emulsions having a ratio of squalene:QS21 in the range of 1:1 to 200:1, are preferred emulsions for use in the present invention. Emulsions having a ratio of squalene:QS21 in the range of substantially 48:1, are also preferred emulsions for use in the present invention. This reduction of one of the components has the surprising effect of qualitatively improving the resultant immune response. Using this adjuvant formulation strong Th2-type responses are maintained, but moreover such formulations elicit an enhanced immune response specifically associated with Th1-type responses, characterised by high IFN-γ, T-cell proliferative and CTL responses.

The present invention also provides a method for producing a vaccine formulation comprising mixing an immunogen and carrier of the present invention together with a pharmaceutically acceptable excipient, such as 3D-MPL.

A most preferred adjuvant for use in the invention is the combination of QS21, 3D MPL and an oil-in-water emulsion, or the combination of 3D MPL and QS21 quenched with cholesterol as described above.

The composition of the invention may be delivered by any suitable delivery means and route of administration, suitably by intramuscular injection.

In one aspect of the present invention, the immunogen and carrier of the present invention may be encapsulated into microparticles such as liposomes. Encapsulation within liposomes is described, for example, by Fullerton, U.S. Patent 4,235,877.

Typically, when 3D-MPL is used, the antigen and 3D-MPL are delivered with alum or presented in an oil-in-water emulsion or multiple oil-in-water emulsions. The incorporation of 3D-MPL is advantageous since it is a stimulator of effector T-cell responses.

Accordingly a preferred embodiment of the present invention provides a vaccine comprising an immunogen and carrier as herein described, in combination with 3D-MPL and a vehicle. Typically the vehicle will be an oil-in-water emulsion or alum.

In a preferred embodiment, the adjuvant for use in the present invention is selected from the group of adjuvants comprising: a monophosphoryl lipid A or derivative thereof, QS21, a mixture of QS21 and cholesterol, and a CpG oligonucleotide. Another preferred adjuvant comprises a monophosphoryl lipid A or derivative thereof, QS21 and tocopherol in an oil-in-water emulsion. Preferably, the monophosphoryl lipid A or derivative thereof is 3D-MPL.

A preferred adjuvant for use in the present invention is a formulation comprising QS21 and an oil-in-water emulsion, wherein the oil-in-water emulsion comprises a metabolisable oil, such as squalene, α-tocopherol and a polysorbate (including polyoxyethylene sorbitan monooleate, TWEEN 80), said emulsions being characterised in that the ratio of the oil:QS21 is in the range of 20:1 to 200:1 (w/w), most preferably substantially 48:1 (w/w). Such a formulation once combined with an antigen or antigenic preparation is suitable for a broad range of monovalent or polyvalent vaccines. Additionally the oil-in-water emulsion may contain polyoxyethylene sorbitan trioleate (SPAN 85). Preferably, the oil-in-water emulsion contains cholesterol.

A preferred adjuvant formulation for use in the present invention is disclosed in PCT application no WO99/11241; which publication is incorporated herein by reference.

A preferred form of 3 De-O-acylated monophosphoryl lipid A is disclosed in PCT application no. 92116556 - SmithKline Beecham Biologicals s.a.

The ratio of QS21 : 3D-MPL (w/w) in a preferred embodiment of the present invention will typically be in the order of 1 : 10 to 10 : 1; preferably 1 : 5 to 5 : 1 and often substantially 1 : 1. The preferred range for optimal synergy is from 2.5:1 to 1:1 3D MPL: QS21. Typically, the dosages of QS21 and 3D-MPL in a vaccine for human administration will be in the range 1 μg - 1000 μg, preferably 10 μg - 500 μg, and most preferably 10-100 μg per dose. Typically the oil-in-water will comprise from 2 to 10% squalene, from 2 to 10% α-tocopherol and from 0.4 to 2% polyoxyethylene sorbitan monooleate (TWEEN 80). Preferably the ratio of squalene: α-tocopherol is equal or less than 1 as this provides a more stable emulsion. Polyoxyethylene sorbitan trioleate (SPAN 85) may also be present at a level of 0.5 - 1%. In some cases it may be advantageous that the vaccines of the present invention will further contain a stabiliser, for example other emulsifiers/surfactants, including caprylic acid (Merck index 10th Edition, entry no.1739), of which Tricaprylin is a particularly preferred embodiment.

Therefore, another embodiment of this invention is a vaccine containing QS21 and an oil-in-water emulsion falling within the desired ratio, which is formulated in the presence of a sterol, preferably cholesterol, in order to reduce the local reactogenicity conferred by the QS21. The ratio of the QS21 to cholesterol (w/w), present in a specific embodiment of the present invention, is envisaged to be in the range of 1:1 to 1:20, substantially 1:10.

The previous emulsions used in PCT application no. WO 95/17210, in particular adjuvants comprising oil-in-water emulsion, MPL and QS21 are preferred adjuvants for use in the present invention as they obviously hold some advantages over conventional non-Th1 inducing adjuvants. It has been observed that formulation of the QS21 into cholesterol containing liposomes may help prevent necrosis occurring at the site of injection. This observation is subject to PCT Application No. PCT/EP96/01464, and the adjuvant disclosed therein, particularly an adjuvant comprising liposome, MPL and QS21 is also a preferred adjuvant for use in the present invention.

In embodiments of the present invention the preferred sterol is cholesterol. Other sterols which could be used in embodiments of the present invention include β-sitosterol, stigmasterol, ergosterol, ergocalciferol and cholesterol. Sterols are well known in the art.

Cholesterol is well known and is, for example, disclosed in the Merck Index, 11th Edn., page 341, as a naturally occurring sterol found in animal fat.

Such preparations are used as vaccine adjuvant systems and once formulated together with antigen or antigenic preparations for potent vaccines. Advantageously they preferentially induce a Th1 response.

The emulsion systems of the present invention preferably have a small oil droplet size in the sub-micron range. Preferably the oil droplet sizes will be in the range 120 to 750nm, and most preferably from 120-600nm in diameter.

A preferred form of 3 De-O-acylated monophosphoryl lipid A is in the form of an emulsion having a small particle size less than 0.2μm in diameter, the method of manufacture of which is disclosed in International Patent Application No. 92/116556.

A particularly preferred adjuvant for use in the present invention is SB62'c, an adjuvant comprising an oil-in-water emulsion and a saponin, wherein the oil is a metabolisable oil, and the ratio of the metabolisable oil:saponin (w/w) is in the range of 1:1 to 200:1 (oil-in-water emulsion low dose; MPL and QS21) described in WO99/11241, the full teaching of which is incorporated herein by reference. Another particularly preferred adjuvant consists of an oil-in-water emulsion such as that described in EP0382271. A further particularly preferred adjuvant is Liposome/MPL/QS21 such as described in EP822831.

Excipients

Suitably the composition of the invention may be combined with appropriate pharmaceutically acceptable vehicles or excipients to form a vaccine formulation. Appropriate vehicles and excipients are well known in the art and include for example water or buffers. Vaccine preparation is generally described in Vaccine Design ("The subunit and adjuvant approach" (eds Powell M.F. & Newman M.J.) (1995) Plenum Press New York).

Peptide synthesis

Peptides used in the present invention can be readily synthesised by solid phase procedures well known in the art. Suitable syntheses may be performed by utilising "T-boc" or "F-moc" procedures. Cyclic peptides can be synthesised by the solid phase procedure employing the well-known "F-moc" procedure and polyamide resin in the fully automated apparatus. Alternatively, those skilled in the art will know the necessary laboratory procedures to

perform the process manually. Techniques and procedures for solid phase synthesis are described in 'Solid Phase Peptide Synthesis: A Practical Approach' by E. Atherton and R.C. Sheppard, published by IRL at Oxford University Press (1989). Alternatively, the peptides may be produced by recombinant methods, including expressing nucleic acid molecules encoding the mimotopes in a bacterial or mammalian cell line, followed by purification of the expressed mimotope. Techniques for recombinant expression of peptides and proteins are known in the art, and are described in Maniatis, T., Fritsch, E.F. and Sambrook et al., Molecular cloning, a laboratory manual, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989).

Nucleic acids

Also forming part of the present invention are portions of nucleic acid which encode the immunogens of the present invention or peptides, mimotopes or derivatives thereof, or recombinant fusion proteins comprising the immunogens. In particular isolated nucleic acid molecules which encode an immunogen of the present invention, preferably together with a carrier, are provided, which may be used for DNA vaccination. Helpful background information in relation to DNA vaccination is provided in "Donnelly, J *et al Annual Rev. Immunol.* (1997) 15:617-648, the disclosure of which is included herein in its entirety by way of reference. In an embodiment of the present invention in which the immunogen is administered in the form of a DNA vaccination, the composition may further comprise a vehicle. Preferably, the vehicle is a gold bead, or comprises a gold bead. Other vehicles or excipients described herein may also be used. The nucleic acid constructs may be formulated within plasmids for delivery.

Therapeutic uses

The formulations of the present invention maybe used for both prophylactic and therapeutic purposes. In a further aspect of the present invention there is provided a vaccine as herein described for use in medicine. Vaccine preparation is generally described in New Trends and Developments in Vaccines, edited by Voller et al., University Park Press, Baltimore, Maryland, U.S.A. 1978.

The vaccine preparations of the present invention may be used to protect or treat a mammal susceptible to, or suffering from a disease, by means of administering said vaccine via systemic or mucosal route. These administrations may include injection *via* the intramuscular, intraperitoneal, intradermal or subcutaneous routes; or *via* mucosal administration to the oral/alimentary, or respiratory tracts.

The vaccine of the present invention is useful in the prevention, treatment and/or amelioration of clinical signs associated with neurological diseases such as multiple sclerosis or Guillain-Barré Syndrome, myasthenia gravis; bowel diseases such as Crohn's disease; and autoimmune-implicated diseases including but not limited to systemic lupus erythematosis, rheumatoid arthritis, thyroiditis including Hashimoto's thyroiditis, pernicious anaemia, Addison's disease, diabetes, dermatomyositis, Sjogren's syndrome, multiple sclerosis, Reiter's syndrome, Graves disease and psoriasis. Most preferably, the vaccine of the present invention may be used in the prevention, treatment and/or amelioration of clinical signs associated with one or more of the following conditions: multiple sclerosis; Crohn's disease; thyroiditis; and rheumatoid arthritis.

In one aspect of the present invention there is provided a method of treating a disease, for example an autoimmune-implicated disorder, by administration of a vaccine according to the present invention.

Dosing regimen

Vaccines may be delivered in any suitable dosing regime, such as a one, two, three or more dose regimes. Following an initial vaccination, subjects may receive one or several booster immunisation adequately spaced. Such a vaccine formulation may be either a priming or boosting vaccination regime; be administered systemically, for example *via* the transdermal, subcutaneous or intramuscular routes or applied to a mucosal surface via, for example, intra nasal or oral routes.

There can, of course, be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

It is possible for the vaccine composition to be administered on a once off basis or to be administered repeatedly, for example, between 1 and 7 times, preferably between 1 and 4 times, at intervals between about 1 day and about 18 months, preferably one month. This may be optionally followed by dosing at regular intervals of between 1 and 12 months for a period up to the remainder of the patient's life. Preferably, following an initial vaccination, subjects will receive a boost in about 4 weeks, followed by repeated boosts every six months for as long as a risk of infection or disease exists. The immune response to the protein of this invention is enhanced by the use of adjuvant and or an immunostimulant.

In an embodiment of the present invention the patient will receive the antigen in different forms in a prime/boost regime. Thus for example an antigen will be first administered as a DNA based vaccine and then subsequently administered as a protein adjuvant base formulation, or vice versa. Once again, however, this treatment regime will be significantly varied depending upon the size and species of animal concerned, the amount of nucleic acid vaccine and / or protein composition administered, the route of administration, the potency and dose of any adjuvant compounds used and other factors which would be apparent to a skilled veterinary or medical practitioner.

The amount of protein in each vaccine dose is selected as an amount which induces an immunoprotective response without significant, adverse side effects in typical vaccinees. Such amount will vary depending upon which specific immunogen is employed and whether or not the vaccine is adjuvanted. Generally, it is expected that each dose will comprise 1-1000μg of protein, preferably 1-500μg, more preferably 1-200μg, more preferably 1-100μg and most preferably 1-50μg. An optimal amount for a particular vaccine can be ascertained by standard studies involving observation of antibody titres and other responses in subjects.

Vaccine preparation is generally described in "Vaccine Design – The Subunit and Adjuvant Approach" 1995, Pharmaceutical Biotechnology, Volume 6, Eds. Powell, M.F., and Newman, M.J., Plenum Press, New York and London, ISBN 0-306-44867-X.

The invention is now illustrated by the following non-limiting examples

Examples

Material and methods

Example 1

Vaccine preparation and immunisation.

Mouse IL-12, histidine-tagged on p35, was prepared as described in Fallarino et al., JI, 1996 156(3): p.1095-1100] This product was coupled to Ova or helper peptides by overnight reaction under cooling with 20 mM glutaraldehyde in 0.1 M phosphate buffer at pH 6. The reaction was stopped by addition of Tris-HCl pH 9 (0.1 M final concentration) and the resulting products dialysed against PBS. For coupling to Ova, a 1/1 molar ratio per IL-12 subunit was used. Synthetic helper peptides selected for strong MHC Class II binding included Pan DR epitope peptide (PADRE) (aKXVAAWTLKAAC), and tetanus peptides

(CQYIKANSKFIGITEL) or (cFNNFTVSFWLRVPKVSASHLE) [see: Alexander et al., Immunity, 1994. 1(9): p. 751-61]. These were coupled in ratios of 5 peptides per IL-12 subunit.

Other complexes were prepared by introducing sulfhydryl groups in IL-12 through reaction with 2-iminothiolane (Traut's reagent) before conjugation to maleimide-activated carriers, including Ova, keyhole limpet Haemocyanin (KLH) or cationised BSA according to the manufacturer protocols (Pierce, IL, USA).

Vaccines were administered s-c or i.m. with one of the following adjuvants: complete Freund's adjuvant (CFA); Liposome/MPL/QS21 (GSK); Immun-Easy Mouse Adjuvant (Qiagen, Valencia, Ca); CpG oligodeoxynucleotide 1826 (5'-TCCATGACGTTCCTGACGTT-3') with phosporothioate modification [Ballas et al., JI 2001 167(9) p4878-86]; and SB62'c, an adjuvant comprising an oil-in-water emulsion and a saponin, wherein the oil is a metabolisable oil, and the ratio of the metabolisable oil:saponin (w/w) is in the range of 1:1 to 200:1 (GSK, as described in WO99/11241, the full teaching of which is incorporated herein by reference).

Example 2

Assessment of anti-IL-12 antibodies.

For detection of anti-IL-12 antibodies by ELISA, Maxisorb Nunc-Immunoplates (Nalge Nunc International,Hereford, U. K.) were coated with IL-12 or BSA as a control (both at 5µg/ml) in 20 mM glycine buffer pH 9.3. After blocking with 1 % BSA in PBS, sera diluted in blocking buffer were added to the plates and incubated at 37 °C for 2 h. After washing, peroxidase-coupled goat anti-mouse IgG (Transduction Laboratories, Lexington KY) followed with Ultra-TMB substrate (Pierce, Rockford, IL, USA) were used to detect bound antibodies.

The specificity of these antisera was further analysed by pre-incubating appropriately diluted samples with IL-12 heterodimers or P40 homodimers (R &D, Minneapolis) both at 1 µg/ml for 2 h before incubation on IL-12-coated plates.

Inhibition of IL-12 activity was measured *in vitro* by testing inhibition of IL-12-induced proliferation of ConA-blasts prepared from C57Bl/6 spleen cells according to Schoenhaut [Schoenhaut et al., JI, 1992. 148(11) p3433-40] Alternatively, 10^4 Baf3 cells transfected with murine IL-12 receptors (a kind gift of Dr. Jean-Christophe Renauld, LICR, Brussels Branch). were put in 96 well plates, in 200 µl DMEM with 10% FCS and proliferation was measured

48h later after addition of tritiated thymidine for the last 16 hours. Inhibition titres were calculated as the reciprocal serum dilution giving 50 % inhibition of 1ng/ml IL-12.

Example 3

Assessment of IL-12 activity in anti-IL-12 immunised mice *in vivo*.

C57Bl/6 mice immunised with IL-12-PADRE or vehicle were treated on 3 consecutive days with 500 ng IL-12. One day after the last injection, blood was collected and IFNγ serum concentration was determined.

Example 4

Induction of experimental allergic encephalomyelitis (EAE).

EAE was induced in SJL and C57Bl/6 mice previously immunised with IL-12-PADRE complexes in an adjuvant comprising an oil-in-water emulsion and a saponin, wherein the oil is a metabolisable oil, and the ratio of the metabolisable oil:saponin (w/w) is in the range of 1:1 to 200:1 (GSK), or with adjuvant only. In SJL, EAE was elicited according to Weinberg [Weinberg, et al., JI, 1999. 162(3) p1818-26], using 150µg proteolipid protein (PLP) peptide 139-151 (HCLGKWLGHPDKF) injected in CFA along with 200 µg *Mycobacterium butyricum* (Difco Lab., Detroit, MI) in 2 x 50µl at the base of the tail and in 2 x 50µl aliquots s.c. in the flanks. In C57Bl/6, 100µg myelin oligodendrocyte glycoprotein (MOG) peptide 35-55 (MEVGWYRSPFSRVVHLYRNGK) was injected in CFA containing 800µg *Mycobacterium butyricum* (2 x 50 µl sc at the base of the tail). Mice were then injected intravenously with 300 ng of *Pertussis toxin* (Calbiochem) in 100 µl PBS containing 1% NMS. The Pertussis toxin injection was repeated after 48h according to the protocol described by Slavin [Slavin et al., Autoimmunity, 1998. 28(2) p109-20]. Disease was evaluated by determination of body weight and EAE scoring according to Heremans [Heremans, et al., Eur Cytokine Netw, 1999. 10(2) p171-80].

Example 5

Determination of antibody responses to PLP peptide.

Anti-PLP IgG1 and IgG2a antibodies were tested on Maxisorb plates coated with PLP peptide at 2µg/ml. After blocking with 1 % BSA, serial serum dilutions were incubated for 2 h and, after washing, anti-IgG1 (LOMG1) or anti-IgG2a (LOMG2a) rat antibodies coupled to HRP (IMEX , Brussels, Belgium) were added. Plates coated with BSA gave negligible signals.

Popliteal lymph nodes collected from 5 to 14 weeks after EAE induction were stimulated *in vitro* with PLP for 72 h and IFNγ was measured by ELISA (Biosource Europe Fleurus Belgium) or bioassay respectively.

Example 6
ELISA

IFNγ concentrations in culture supernatant were determined by sandwich ELISA. Supernatants and appropriate cytokine standards (PharMingen, San Diego, CA) were used in threefold serial dilutions. Purified and biotinylated antibodies were purchased from PharMingen. Detection was performed with alkaline phosphatase-coupled streptavidin (Southern Biotechnology, Birmingham AL). Detection limits for IFNγ are 46pg/ml. Serum samples and appropriate immunoglobulin standards (Southern Biotechnology, Birmingham, AL) were used in 3-fold serial dilutions. Detection limits were 5 ng/ml for IgG1 and 0.1ng/ml for IgG2a. Total IgE was determined with mAbs 84.1C for coating and alkaline phosphatase labeled EM95.3 for detection. The detection limit for IgE was 10 ng/ml.

Results

Example 8
Induction of anti-IL-12 auto-antibodies.

Immunisation of mice with mouse IL-9 coupled to Ova with glutaraldehyde and emulsified in CFA triggers the production of anti-IL-9 auto-antibodies, leading to efficient suppression of IL-9 activities *in vivo* [Richard, et al., PNAS USA, 2000. 97 p767-772.]. Similar attempts made with IL-12 were, however, not successful. We therefore changed the adjuvant to Liposome/MPL/QS21 (GSK). This resulted, in C57Bl/6 mice, in the production of significant antibody titres as assessed by ELISA (Figure 1A) and inhibition of IL-12-induced proliferation of ConA-activated T cells (Figure 1B). The specificity of this inhibition was demonstrated by undiminished responses of similarly prepared blasts to IL-2.

These results highlighted the importance of the adjuvant for such immunisations. We therefore tested several other products with immune-stimulating properties, including SB62'c (GSK); ImmunEasy a commercial adjuvant based on CpG from Qiagen; and CpG 1826, a phosporothioate-modified DNA with CpG motifs. As shown in Figure 2A, SB62'c induced responses that were approximately ten times better than those obtained with adjuvants not

containing QS21 or MPL. In the same Figure are shown results obtained with IL-12 coupled to PADRE and Tetanus helper peptides. These complexes gave results essentially similar to those obtained with IL-12-Ova, indicating that an effective vaccine could be obtained by direct addition of the helper peptides.

Numerous methods, often more refined than that using glutaraldehyde, have been developed for protein cross-linking. One is to introduce free sulfhydryl groups in the protein of interest, which ensures its reaction with maleimide-substituted carriers. Such complexes were prepared with IL-12 by reacting the protein with Traut's reagent before cross-linking to maleimide-substituted Ova, KLH or cBSA. For comparison, mice were similarly immunised with IL12-OVA complexes made with glutaraldehyde. As shown in Figure 2B, IL-12 coupled to Ova with both methods gave similar results. However, the other carriers were ineffective. These results prove that mere injection of IL-12 coupled to foreign carrier proteins, even with potent adjuvants, will not systematically break self-tolerance, but that proper combinations of carrier and adjuvant are required to induce significant responses.

Analysis of the kinetics of anti-IL-12 vaccination showed that neutralizing titers were observed only after multiple injections (usually 4 or 5), titers often continued to increase for several weeks after the last immunization and persisted for unlimited periods of time (Figure 2 C).

Example 9
Specificity of anti-IL-12 antibodies

The complexes used for immunisation were made with recombinant IL-12p70 (p40-p35 heterodimers). Since the antisera showed antibody binding to IL-12 p70 coated plates, competition experiments were carried out to analyse their relative interactions with p40 versus p70. Appropriately diluted sera were incubated with IL-12 p70 or p40 homodimers prior to transfer to IL-12-coated plates. Both P40 dimers and IL-12 heterodimers had equivalent inhibitory activities, indicating that most of the anti-IL-12 antibodies reacted with the p40 subunit. (Figure 3).

Example 10

Anti-IL-12 vaccinated mice no longer respond to IL-12 *in vivo*.

Repeated administration of IL-12 to normal mice induces elevated IFNγ levels in the serum [Gately, et al., Int Immunol, 1994 6(1) p157-67]. We used this procedure to evaluate the functional efficacy of anti-IL-12 vaccination. As shown in Figure 4, after injection of IL-12 for

3 consecutive days, IFNγ levels were in the nanogram/ml range in control mice but remained undetectable (< 0.03 ng/ml) in anti-IL-12 -vaccinated animals.

Example 11

Anti-IL-12 vaccine impairs EAE-induction.

SJL mice were immunized with IL-12-PADRE peptides or vehicle in the presence of SB62'c adjuvant before induction of EAE by immunization with PLP peptide. After four injections, reciprocal anti-IL-12 neutralizing antibody titers were 6,513 ± 2,012. As shown in Figure 5, EAE symptoms became apparent in control adjuvant-treated mice from day 12, peaked around day 20 (one of the animals died on day 17), then gradually subsided but were still detectable after one month in one third of the animals. In anti-IL-12 vaccinated mice only minimal signs of disease were detected and all mice survived. Moreover, body weight drop, another feature of PLP-induced EAE, was completely absent in the vaccinated animals. Of note, administration of SB62'c by itself had a slight protective activity as compared to mice receiving simply PBS.

The protective effect of IL-12 vaccination was expected to imply suppression of IFNγ production and changes in anti-PLP antibody IgG subclasses.

Analysis of anti-PLP IgG1 and IgG2a antibodies, showed that there was a clear increase in IgG1 anti-PLP titres ($p < 0.001$) and a reduction in IgG2a that was at the limit of statistical significance ($p = 0.052$) (Figure 6A). Together, these results clearly show that IL-12 vaccination induces fundamental changes in anti-PLP response.

The former hypothesis was tested with lymph node cells stimulated *in vitro* with PLP peptide. IFNγ concentrations were 430 ± 139 pg/ml in 8 IL-12 vaccinated mice and 1939 ± 634 in 9 SB62'c controls ($p = 0.0079$ Mann-Whitney). Popliteal lymph nodes collected from 5 to 14 weeks after EAE induction (8 and 9 mice in IL-12-PADRE and SB62'c groups) were stimulated *in vitro* with PLP peptide. IFNγ concentrations were measured after 3 days (Figure 6B).

To test whether anti-IL-12 vaccination would also prevent the more aggressive form of EAE induced by immunisation with MOG peptide, C57Bl/6 mice vaccinated with IL-12-PADRE complexes in the presence of SB62'c before immunisation with MOG had reciprocal inhibition titres of 19,577 ± 3,792. Extremely elevated EAE scores were noted in the control group and 2 of the 15 mice in this population died after 26 and 33 days respectively. Anti-IL-

12 vaccinated mice showed a 2-3 day delayed onset and reduced maximal disease scores as well as body weight losses. Moreover, none of these mice died and 11/15 showed complete recovery, which occurred only in 4/15 controls ($p = 0.027$ by Fisher's statistics). Also in MOG-induced EAE was there a protective effect of SB62'c as compared to PBS-treated mice. This was particularly striking for body weight recovery, which was accelerated by more than a week.

To further evaluate the potency of our vaccine and to compare it with results obtained by administration of anti-IL-12 antibodies, one additional groups was included in the former MOG experiment. This group received repeated injections of C17.8, a rat anti-p40 antibody, which has previously been shown to inhibit EAE in NOD mice [Ichikawa et al., J Neuroimmunol, 2000. 102(1) p56-66]. As shown in Table 1, mean weight losses and EAE scores in C57Bl/6 mice were reduced by these antibodies to similar levels as those observed with the IL-12-PADRE vaccine. The figures correspond to 14 measurements made from day 9 to day 51 in 15 C57Bl/6 mice per group. The probabilities were calculated by Mann-Whitney non-parametric statistics.

Table 1

	Weight	P	EAE score	P
IL-12-PADRE-SB62'c	95 +/- 1.68		1.028 +/- 0.229	
SB62'c	85.6 +/- 9	0.0045	2.086 +/- 0.33	0.023
C17.8	90.53 +/- 1.94		1.16 +/- 0.138	
PBS	81.6 +/- 3.12	0.0094	2.257 +/- 0.357	0.009

Claims

1. An immunogenic or vaccine composition comprising:
   (a) an immunogenic component comprising
      (i) an immunogen comprising IL-12, IL-23, or a subunit or component thereof; and
      (ii) a carrier; and
   (b) an adjuvant comprising one or more of cholesterol; oil-in-water emulsion; oil-in-water emulsion low dose; tocopherol; liposome; QS21; and 3 D-MPL.

2. An immunogenic or vaccine composition according to claim 1 in which the immunogen comprises the P35 subunit of IL-12.

3. An immunogenic or vaccine composition according to claim 1 in which the immunogen comprises the P40 subunit of IL-12 or IL-23.

4. An immunogenic or vaccine composition according to claim 2 or 3 in which the immunogen comprises at least one surface epitope of P35 or P40.

5. An immunogenic or vaccine composition according to claim 1 in which the carrier comprises one or more of: Keyhole Limpet Haemocyanin (KLH); bovine serum albumin (BSA); tetanus toxin (TT), diphtheria toxin (DT); Domain 1 of Fragment C of TT; the translocation domain of DT; Hep B core protein; PADRE; P2; and P30.

6. An immunogenic or vaccine composition according to any preceding claim in which the immunogen is coupled to the carrier by direct covalent coupling.

7. An immunogenic or vaccine composition according to any of claims 1 to 5 in which the immunogen is coupled to the carrier in the context of a recombinant fusion molecule.

8. An immunogenic or vaccine composition according to any preceding claim in which the adjuvant comprises liposome, MPL and QS21.

9. An immunogenic or vaccine composition according to any of claims 1 to 7 in which the adjuvant comprises oil-in-water emulsion; MPL and QS21.

10. An immunogenic or vaccine composition according to any of claims 1 to 7 in which the adjuvant comprises oil-in-water emulsion low dose; MPL and QS21.

11. An immunogenic or vaccine composition according to any of claims 1 to 7 in which the adjuvant comprises oil-in-water emulsion.

12. A immunogenic or vaccine composition comprising the immunogenic composition as described in any preceding claim in combination with a pharmaceutically acceptable excipient, adjuvant or vehicle.

13. A process for the manufacture of a composition according to claim 12 comprising mixing the immunogenic composition as described herein with a pharmaceutically acceptable excipient, adjuvant or vehicle.

14. A method of preventing or treating a disease or disorder, in particular an autoimmune-implicated disease by administration of an immunogenic composition or vaccine composition according to any of claims 1 to 12.

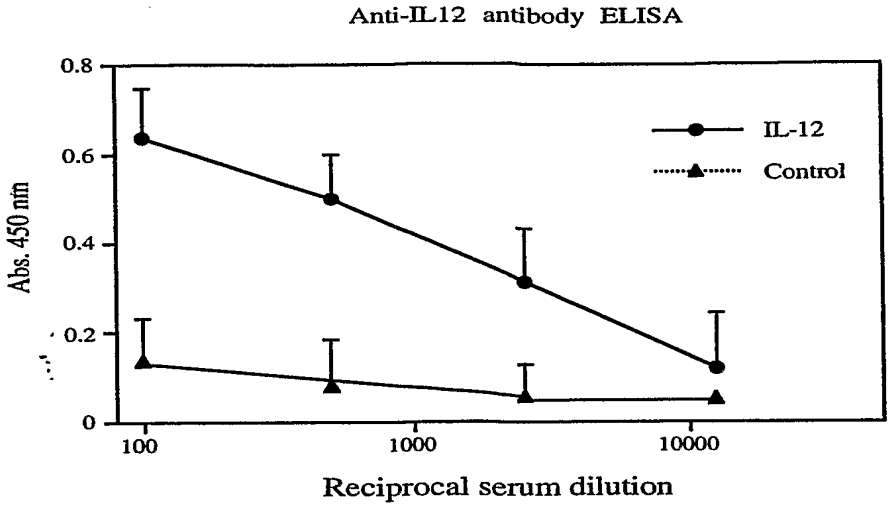
15. Use of an immunogenic composition or vaccine composition according to any of claims 1 to 12, in the manufacture of a vaccine for the prevention, therapy or treatment of a disease or disorder, in particular an autoimmune-implicated disease or disorder.

16. A method or use according to claim 14 or 15, in which the composition is for prevention, therapy or treatment of a disease or disorder of a mammal.

17. A method or use according to claim 16, in which the composition is for prevention, therapy or treatment of a disease or disorder of a human.

18. A method or use according to claim 16, in which the composition is for prevention, therapy or treatment of multiple sclerosis; Crohn's disease; thyroiditis; or rheumatoid arthritis

Fig. 1



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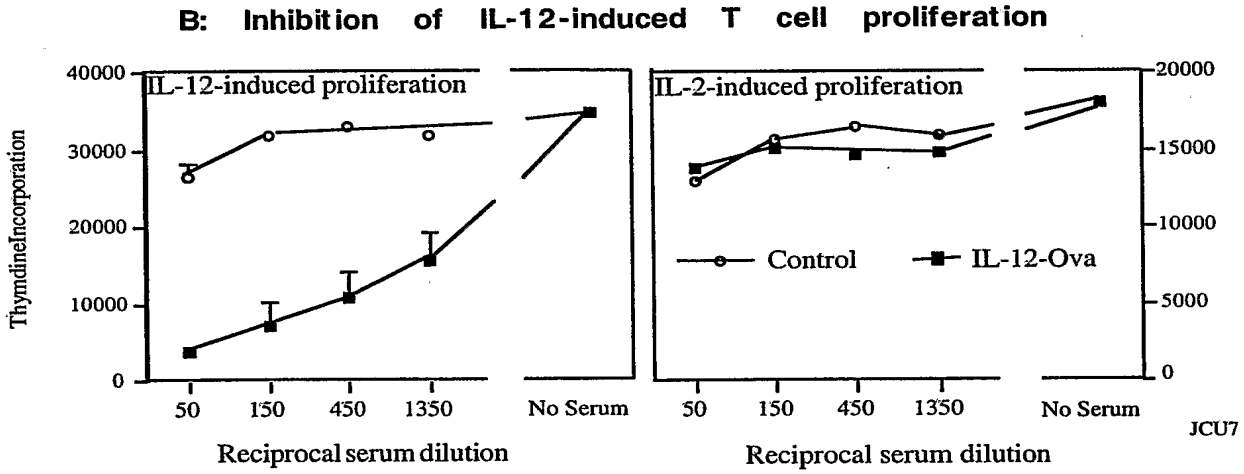


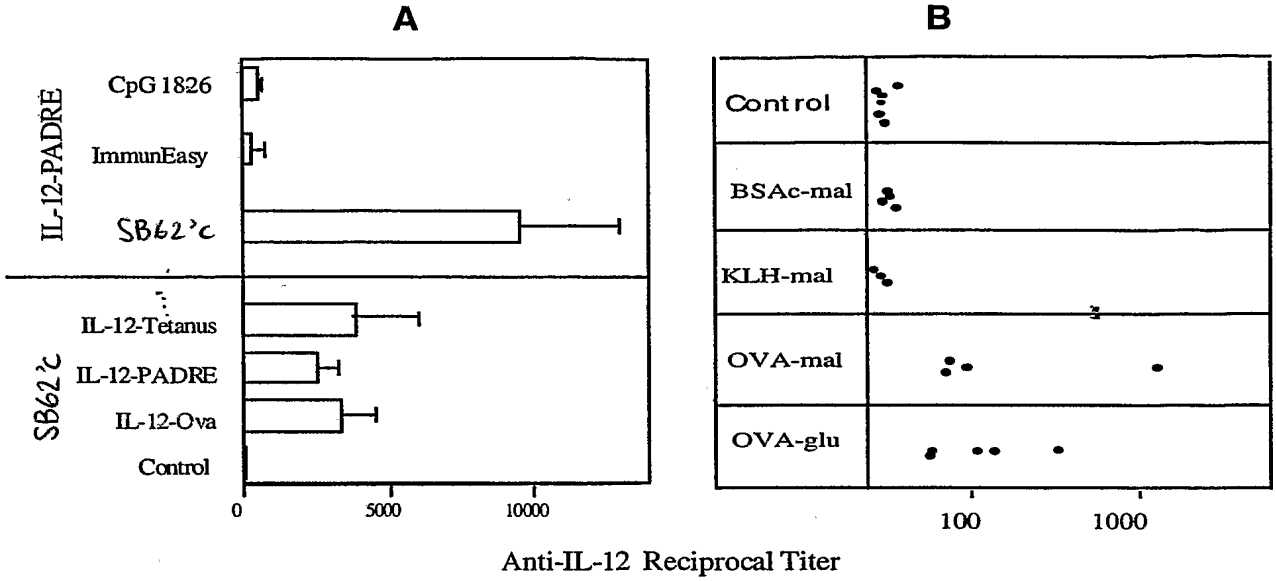
JCU7

Fig.1 A: ELISA plates coated with IL-12 were incubated with dilutions of sera from C57Bl/6 mice immunized with IL-12-Ova or irrelevant Ova complexes in the presence of DQS21/MPL. Bound antibodies were detected by reaction with HRP-conjugated goat anti-mouse Ig followed by peroxidase substrate.

Fig.1 B: Inhibition of IL-12-induced proliferation. Con-A blasts were incubated with IL-12 or IL-2 in the presence of control or anti-IL-12-Ova sera. After 48 h, thymidine incorporation was determined (mean ± SE for 5 mice/group).

Fig.2

Induction of anti-IL-12 autoantibodies with different adjuvants and carriers



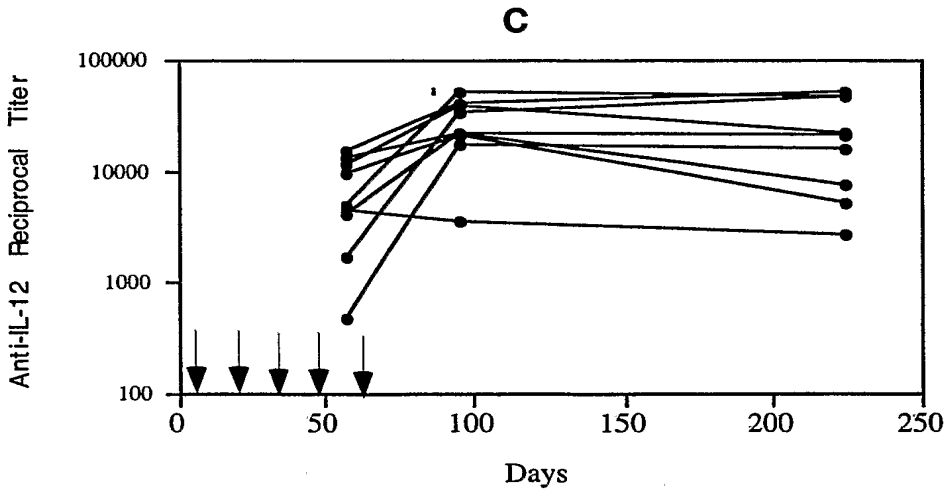


Fig.2: Features of anti-IL-12 auto-antibody responses.

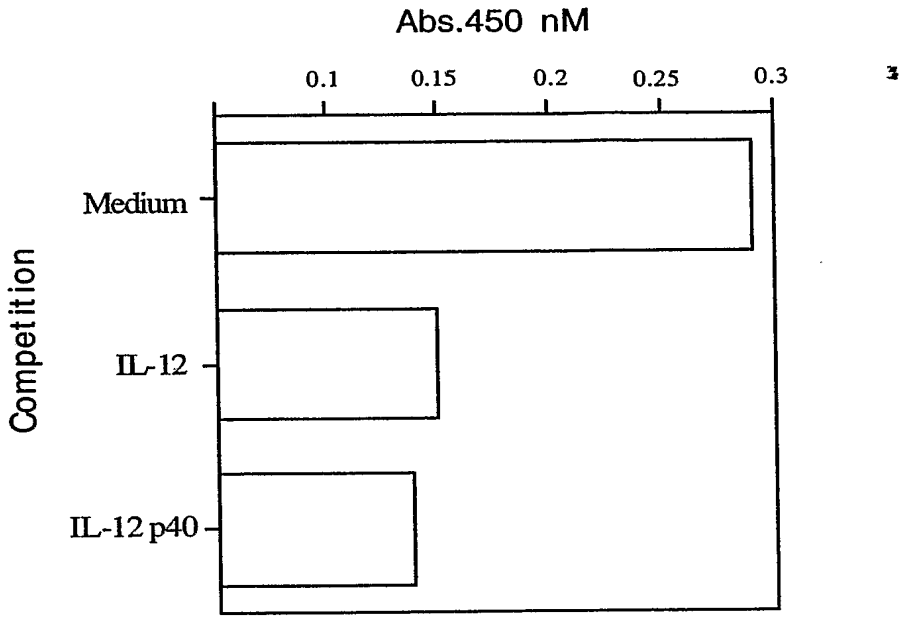
A: Importance of adjuvants. C57Bl/6 mice were immunized with IL-12 coupled to Ova or T-helper peptides (PADRE or Tetanus) in the presence of different adjuvants (JCU44-46). IL-12 inhibitory activities were tested on IL-12-R transfected BaF3 cells.

B: Importance of carrier components: C57Bl/6 mice were immunized with IL-12 coupled to Ova with glutaraldehide or to Ova, KLH or cBSA through maleimide-sulfhydril chross-linking.

C; Persistence of anti-IL-12 antibodies in the 6/6 C57Bl/6 mice immunized with IL-12-PADRE complexes (JCU42).

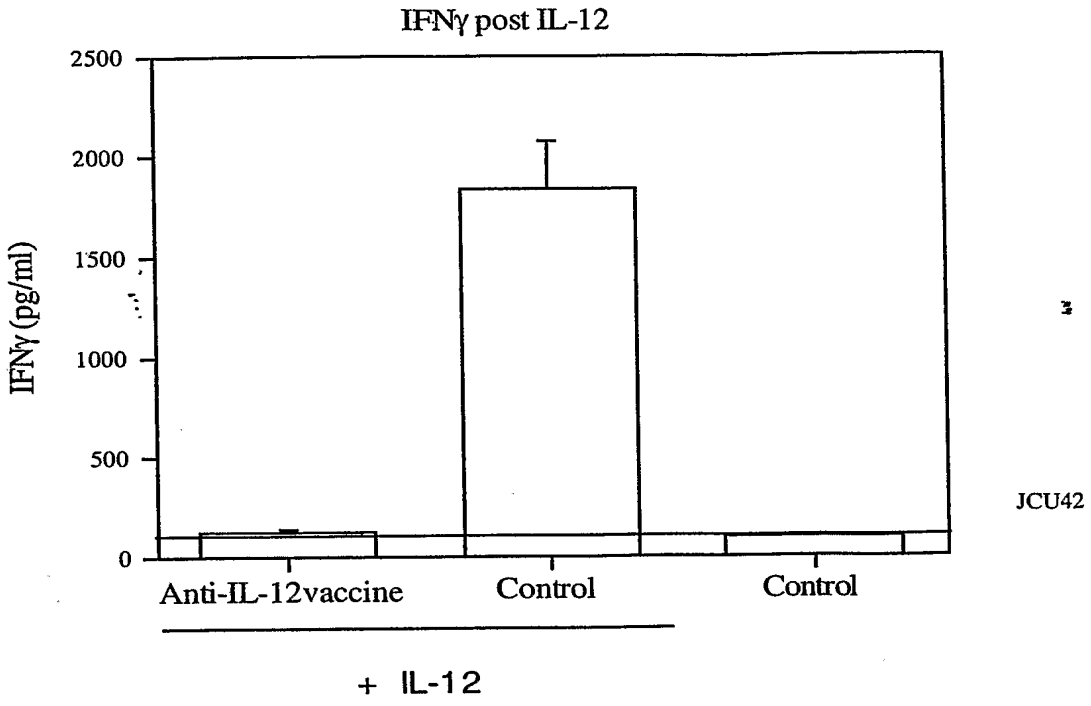
Fig.3

Binding of anti-IL-12 sera to IL-12-coated plate: competition with IL-12 or p40 dimers



Sera from mice vaccinated with IL-12-PADRE complexes were preincubated with IL-12 heterodimer or IL-12 p40 homo-dimers before transfer to IL-12-coated plates. Bound antibodies were then detected with goat anti-mouse Ig.

Fig.4



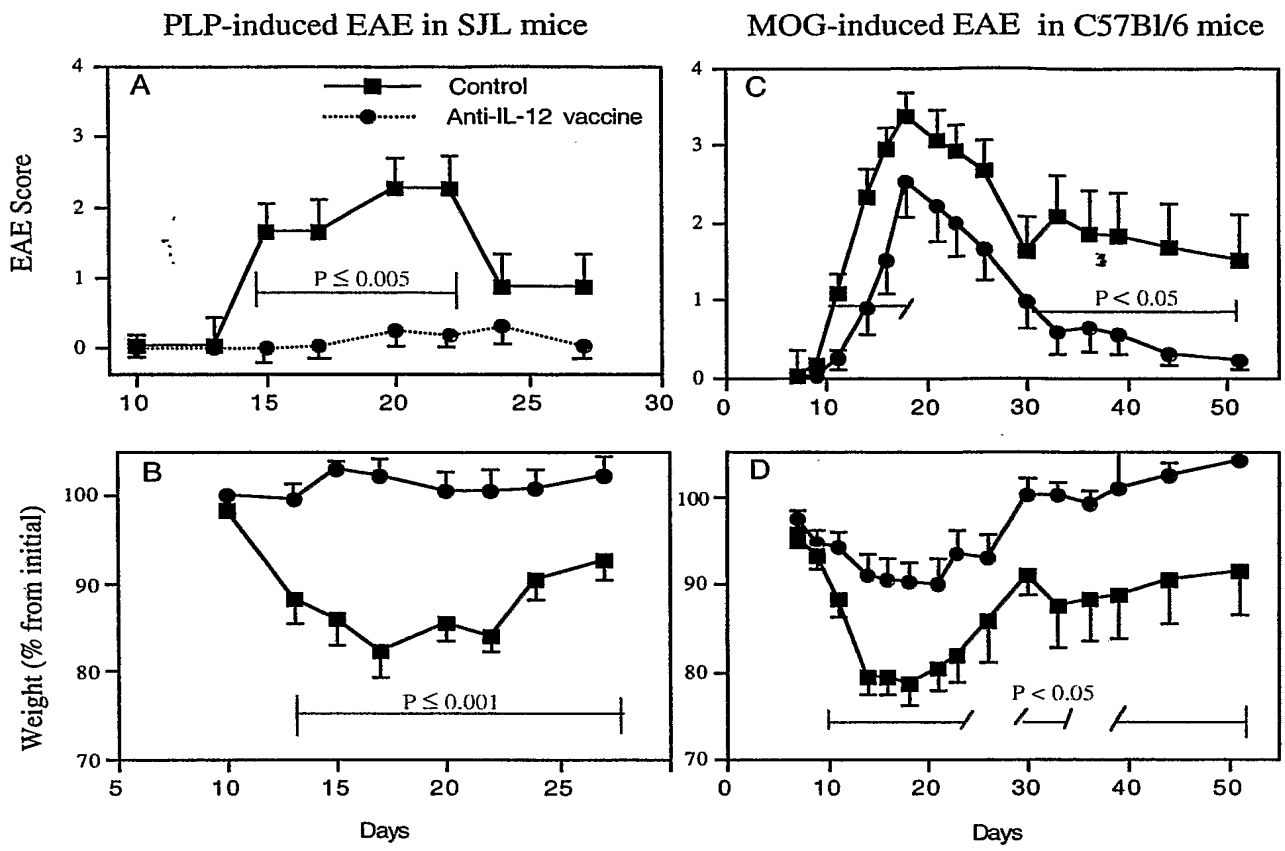
JCU42

Inhibition of IFNγ induction by IL-12 in anti-IL-12 vaccinated mice.

C57Bl/6 mice vaccinated with IL-12-PADRE complexes in SB62c adjuvant were treated with 500 ng IL-12 for 3 consecutive days.

24 h after the last injection, IFNγ concentrations were measured in the serum.

Fig.5



Groups of 13 SJL mice (A and B) previously vaccinated with IL-12-PADRE in SBA2'c or treated with adjuvant only were immunized with PLP peptide for EAE induction.
Similarly vaccinated of control groups of 15 C57Bl/6 mice (C and D) were vaccinated with MOG encephalitogenic peptide. Mean EAE scores and body weight are shown. The differences in both readouts for SJL mice was highly significant ($p < 0.003$ at any time point (Mann-Whitney)). For MOG-induced EAE, the differences in body weight were significant ($p < 0.5$) at all time points except on day 26 ($p = 0.06$). For MOG-induced-disease, EAE scores showed significant differences on days 11, 14 and from day 36 until the end of the experiment. Weight loss was significantly reduced on days 11,14,16, 18, 21, 23, 30, 33 and 36.

Fig. 6

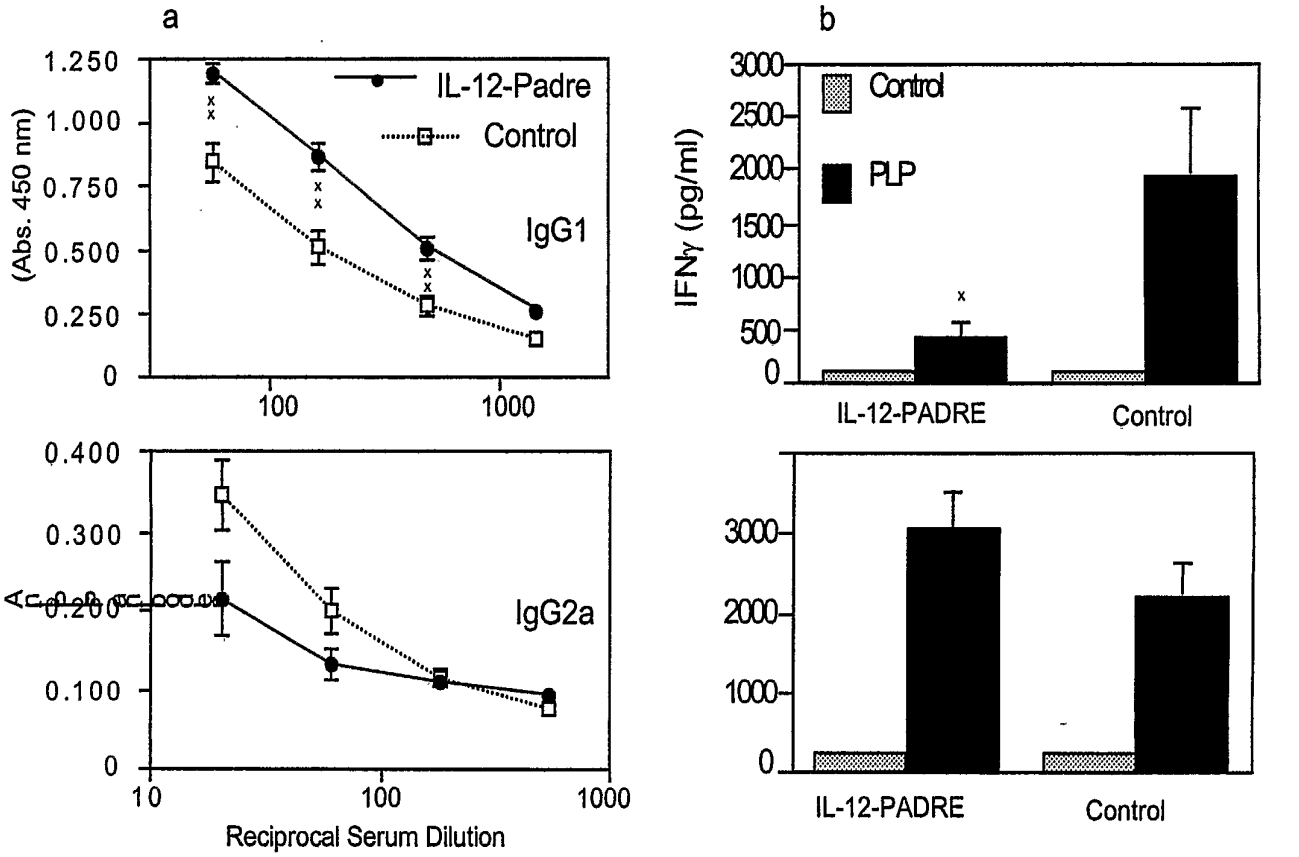
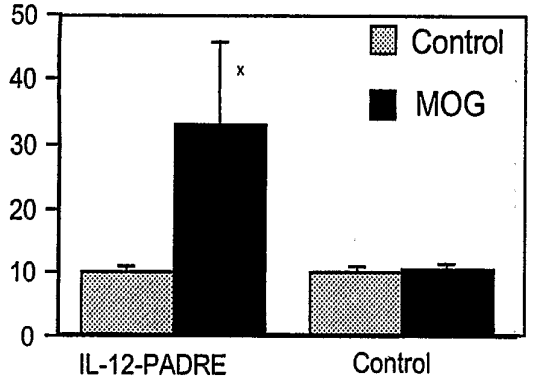
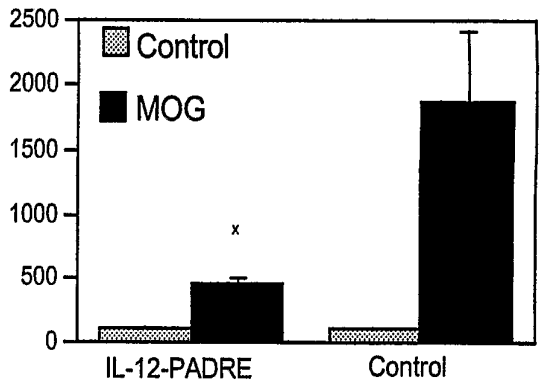

a
b
1.250
1.000
0.750
0.500
0.250
0
(Abs. 450 nm)
IL-12-Padre
Control
IgG1
100
1000
0.400
0.300
0.200
0.100
0
IgG2a
10
100
1000
Reciprocal Serum Dilution
3000
2500
2000
1500
1000
500
0
IFNγ (pg/ml)
Control
PLP
IL-12-PADRE
Control
3000
2000
1000
0
IL-12-PADRE
Control

Fig. 6 c


50
40
30
20
10
0
Control
MOG
x
IL-12-PADRE
Control


2500
2000
1500
1000
500
0
Control
MOG
x
IL-12-PADRE
Control